Full title

Liver steatosis is a risk factor for hepatotoxicity in patients with inflammatory bowel disease treated with azathioprine

Short title/'running head'

Hepatotoxicity in IBD patients on azathioprine

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Disclaimers

None

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Conflict of interest

None declared

Abstract

Background

The literature demonstrates that hepatic steatosis reduces the tolerance of immunosuppression in people with inflammatory bowel disease (IBD). It also shows that elevated methylmercaptopurine (MMP) may-be responsible for thiopurine induced hepatitis. We sought to investigate the relationship between hepatic steatosis, MMP and alanine transaminase (ALT). Our hypothesis was that patients with hepatic steatosis would develop an ALT rise at a lower level of MMP than patients without steatosis.

Methods

To investigate this we performed a retrospective review of patients started on azathioprine treatment at University Hospitals Bristol NHS Foundation Trust between 2014 and 2017. There were 600 patients in total. 121 patients met our inclusion criteria which were at least one ultrasound scan commenting on the appearance of the liver, liver function tests (LFTs) at commencement of azathioprine and LFTs and an MMP level between 6 and 8 weeks after starting treatment.

Results

Of 121 patients included in our study, 40 patients (33%) were identified as having radiological hepatic steatosis on ultrasound imaging and 81 patients had no evidence of steatosis. We found a positive association between MMP levels and change in alanine transaminase (ALT) in patients with fatty liver (*P*<.001) but not in those with a normal liver on ultrasound imaging. We also found that patients with hepatic steatosis had higher MMP levels than those without steatosis (*P*=.03) despite being on lower doses of azathioprine per kilogram of body weight.

Conclusions

We conclude that the higher levels of MMP are a risk factor for hepatitis in patients with hepatic steatosis but not in those with a normal liver, rejecting our hypothesis. In addition, patients with hepatic steatosis tend to produce more MMP.

Keywords

Inflammatory bowel disease

Liver steatosis

Azathioprine

Hepatotoxicity

Risk factor

Introduction

Liver pathology in inflammatory bowel disease (IBD) is common and has been reported in 10-20% of patients with IBD [1,2]. Besides primary sclerosing cholangitis the two most frequent causes of deranged liver function tests in IBD are drug induced hepatotoxicity and non-alcoholic fatty liver disease [1].

Drug induced hepatotoxicity in IBD is often due to thiopurines (azathioprine and 6-mercaptopurine) [3]. These drugs have been reported to induce liver injury in up to 17% of patients [4].

Azathioprine is a prodrug which rapidly degrades to 6-mercaptourine (6MP) in the body via a non-enzymic process. Three enzymes then compete to metabolise 6MP: xanthine oxidase (XO), thiopurine methyltransferase (TPMT) and hypoxanthine phosphoribosyltransferase (HPRT). It is HRPT that is responsible for the production of the active metabolites of thiopurines, the thioguanine nucleotides (TGNs), which interfere with DNA synthesis and exert the therapeutic effect (Fig 1)[5]. TPMT, leads to the production of methylmercaptopurine (MMP) which has been implicated in the hepatotoxic effects of thiopurines [4].

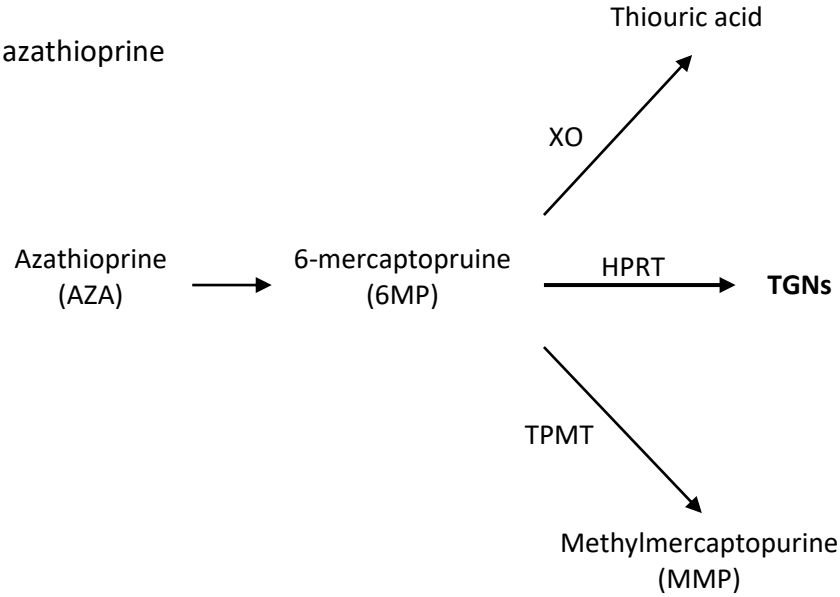
The prevalence of non-alcoholic fatty liver disease (NAFLD) is increasing and has been reported as 20-30% in western populations [6]. It is also being increasingly recognised in the IBD population with a similar prevalence of 23% being seen in this group [7,8]. Although diabetes mellitus and obesity are the predominant risk factors it has been suggested that corticosteroid use and IBD are also independently associated with developing NAFLD [7,9].

NAFLD has been shown to significantly alter the metabolism of many drugs which may lead to altered pharmacokinetics and increased risk of adverse drug reactions [10]. It has previously been reported that hepatic steatosis is a risk factor for developing hepatotoxicity in patients with IBD on immunosuppression [11]. With treatment options for IBD limited, understanding the mechanisms behind drug intolerance is of significant importance in guiding optimal medical treatment.

This study looks at the development of azathioprine induced hepatotoxicity in IBD patients with hepatic steatosis, and the role of MMP level.

Figure 1

Metabolism of azathioprine



Methods

We performed a retrospective review of the medical records of patients started on azathioprine for IBD at University Hospitals Bristol NHS Foundation Trust between June 2014 and May 2017. The medical records of patients identified were analysed on the basis of meeting the inclusion criteria of: (i) at least one abdominal ultrasound commenting on the appearance of the liver, (ii) liver function tests at the commencement of treatment with azathioprine and (iii) liver function tests and an MMP level at 6-8 weeks after starting treatment.

An abdominal ultrasound scan carried out within 5 years of commencing treatment was used to identify hepatic steatosis. Ultrasound reports commenting on the appearance of the liver were considered and results categorised as either showing radiological evidence of steatosis or no steatosis. Patients with evidence of liver cirrhosis, other liver pathologies or ultrasound reports not clearly commenting on the liver were excluded (Fig 2).

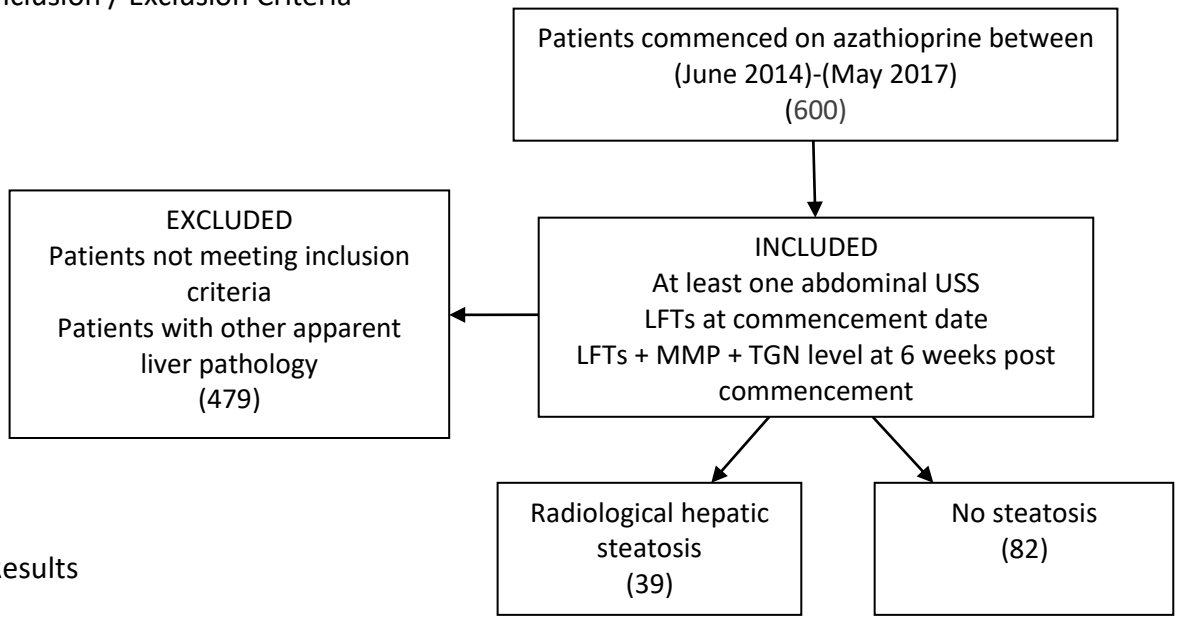
Liver function tests were measured at commencement of azathioprine and at 6-8 weeks after starting treatment. A change in the level of alanine transaminase (ALT) over this 6 week period was calculated and used as a marker of hepatotoxicity. MMP and TGN levels were also measured at between 6 and 8 weeks.

The primary outcome measure was the change in ALT level from commencement of azathioprine in the 'steatosis' versus 'no steatosis' groups and how that related to MMP level. The secondary outcome measure was whether or not MMP levels were different in the 'steatosis' vs 'non steatosis' groups.

Variables recorded included sex, age and weight, disease entity and presence of diabetes mellitus as a comorbidity.

Figure 2

Inclusion / Exclusion Criteria



Results

A total of 121 patients with IBD were suitable for inclusion in the study. Of these 60 (49.6%) were male and 61 (50.4%) were female with a median age of 36 years (interquartile range, IQR, 23-49, range of 9-77 years). All patients were on treatment with azathioprine. Of the 121 patients included 88 (72.7%) had Crohn's disease, 22 (18.2%) had ulcerative colitis and 11 (9.1%) had indeterminate colitis and. The mean weight of included patients was 73.3kg (standard deviation, SD, 20.6kg, range 24.8-157.2kg) and 3 patients (2.5%) had diabetes mellitus as a comorbidity. Body mass index (BMI) was only available for 97 of 121 included patients. The mean dosing of azathioprine was 1.88mg/kg (1.95mg/kg in the no steatosis group and 1.75mg/kg in the steatosis group).

All included patients had an abdominal ultrasound scan within 5 years of commencing azathioprine. Ultrasound reports revealed radiological evidence of hepatic steatosis in 39 patients (32.2%).

The primary outcome measures were change in ALT and MMP level. Change in ALT was measured from commencement of azathioprine to 6 - 8 weeks after starting treatment, the mean change in ALT in patients with hepatic steatosis was 8.2U/L (SD 13.9). The median MMP level at 6 - 8 weeks after commencing azathioprine was 1151 pmol /8x10^8 cells (IQR 471-3109 pmol /8x10^8 cells). This data is shown in Table 1.

To determine the interaction between hepatic steatosis, hepatotoxicity and MMP level, we compared the change in ALT with the MMP level at 6 weeks in the steatosis vs no steatosis groups. Both subgroups were comparable with respect to gender and disease entity. However, the mean weight of patients in the steatosis group was 89.3.0kg (SD 20.1kg) compared with 65.8kg (SD 16.0kg) in the no steatosis group. Also, the 3 patients with diabetes mellitus were all within the hepatic steatosis subgroup.

Using a Wilcoxon rank-sum test, our results found weak evidence of higher MMP levels at 6 weeks in patients with hepatic steatosis compared to those without steatosis (median MMP = 1859 pmol /8x10^8 cells vs 967 pmol /8x10^8 cells for those with and without steatosis respectively, P= .04). The median TGN level was 294 6-TGN/8x10 (IQR 167-424). The median TGN level in the hepatic steatosis group was 315 6-TGN/8x10 (IQR 174-509 6-TGN/8x10) compared to 294 6-TGN/8x10 (IQR 167-3946-TGN/8x10) in patients with no evidence of steatosis on ultrasound (P=0.40).

Linear regression found strong evidence of an interaction between the presence of hepatic steatosis and MMP levels. ALT levels for patients with hepatic steatosis increased by 0.26 U/L per 100 pmol/8x10^8 increase in MMP compared with patients without hepatic steatosis (95% CI 0.10 to 0.41, P=0.002).

Graph 1 displays the change in ALT with increasing MMP level in patients with an ultrasound consistent with fatty liver disease versus those with a radiologically normal liver on ultrasound. The respective lines of best fit show the positive correlation between rising MMP levels and change in ALT in patients with radiological steatosis, but not in patients with a normal liver.

Table 1

Overview of all patients (n=121)	
Demographic data	
Age (years)*	36 (23-49)
Male [n (%)]	60 (49.6)
Azathioprine dose (mg/kg)^	1.88
Metabolic profile	
Weight (kg)^	73.3 (20.6)
BMI (kg/m2)^	26.6 (6.7)
Diabetes mellitus [n (%)]	3 (2.5%)
Disease entity [n (%)]	
Crohn's disease	88 (72.7%)
Indeterminate colitis	11 (9.1%)
Ulcerative colitis	22 (18.2%)
Primary outcomes	
Steatosis [n (%)]	39 (32.2%)
Change ALT (U/L)^	2.1 (11.1)
MMP level (pmol /8x10^8 cells)*	1151 (471-3109)
TGN level (6TGN/8x10)*	294 (167-424)

*median (25%-75%)

^mean (standard deviation)

Table 2

Hepatic Steatosis			
	No steatosis	**Hepatic steatosis**	***P*-value**
Total [n (%)]	82 (67.8)	39 (32.2)	
Demographic data			
Age (years)	31 (22-42)	47 (31-56)	<0.001*
Male [n (%)]	42 (51%)	19 (49%)	
Female [n (%)]	40 (48%)	20 (52%)	
Gender			0.90**
Azathioprine dose (mg/kg)^	1.95	1.73	
Metabolic profile			
Weight (kg)^	65.8 (16.0)	89.3 (20.1)	<0.001^{+}
BMI (kg/m^2)^	23.6 (3.9)	31.9 (7.3)	<0.001^{+}
Diabetes mellitus [n (%)]	0 (0)	3 (8%)	0.01**
Disease entity [n (%)]			
Crohn's disease	58 (71%)	30 (77%)	
Indeterminate colitis	7 (9%)	4 (10%)	
Ulcerative colitis	17 (21%)	5 (13%)	
Primary outcomes			
Change ALT (U/L)^	-1.20 (7.50)	8.19 (13.9)	<0.001^{+}
MMP level (pmol /8x10^8 cells)*	967 (393-2857)	1859 (762-4500)	0.04*
TGN level (6TGN/8x10)*	294 (167-397)	315 (174-509)	0.40*

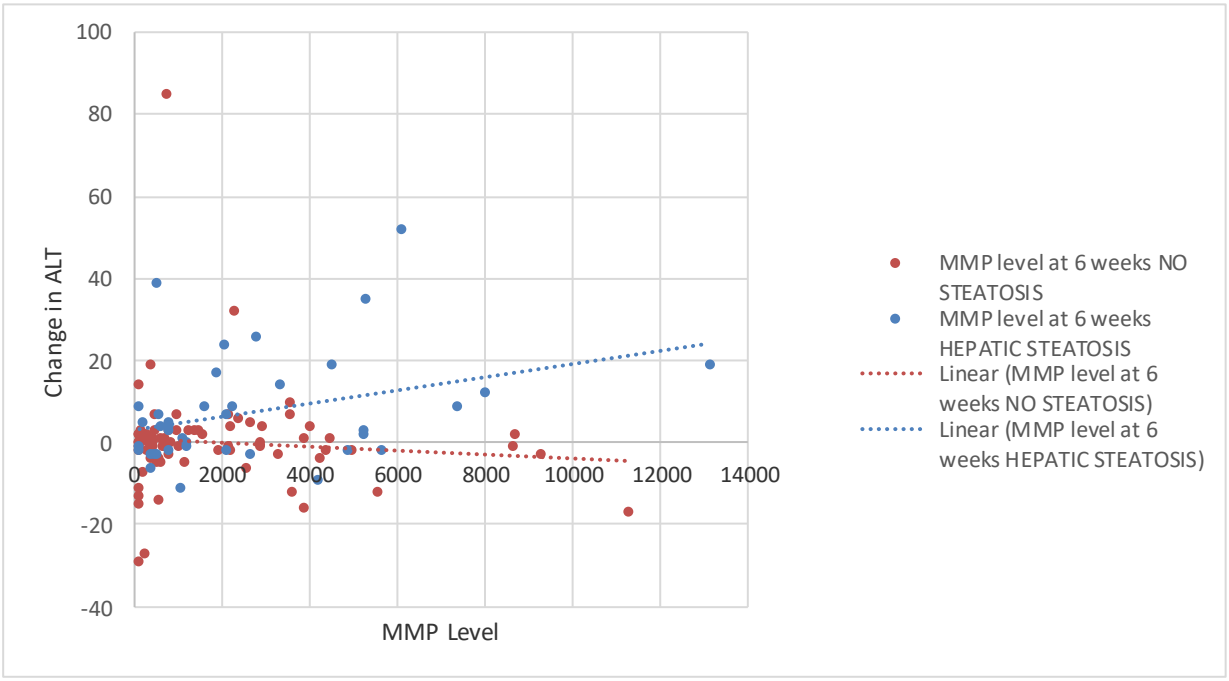
*median (25%-75%)

^mean (standard deviation)

*P value from Wilcoxon's rank sum test , **P values from Chi-square test, $^{+}$P values from t-test

Graph 1

Change in ALT vs MMP level in hepatic steatosis and no steatosis groups



Discussion

The literature suggests that people with inflammatory bowel disease (IBD) and hepatic steatosis do not tolerate the immunosuppression used in IBD [10]. However, previous studies have not measured MMP levels. Our initial hypothesis was that people with hepatic steatosis may suffer an elevation in ALT at a lower level of MMP than people without steatosis.

We performed a retrospective review of IBD patients started on azathioprine treatment at the University Hospitals Bristol NHS Foundation Trust between 2014 and 2017. There were 600 patients in total. We included 121 patients who met the following criteria: i) at least one abdominal USS with specific reference to the appearance of the liver and ii) LFTs taken at the time of commencing azathioprine and then LFTs and MMP between 6 and 8 weeks post commencement.

The main findings of our study were that there is a positive association between MMP levels and a change in alanine transaminase (ALT) in patient with fatty liver on ultrasound (P<.001) but not in patients with a radiologically normal liver, refuting our initial hypothesis. Also, it appears that patients with hepatic steatosis had higher MMP levels than patients without steatosis (P=.04). Together, this suggests that a fatty liver is less tolerant of an elevated MMP and tends to produce more MMP.

The prevalence of non-alcoholic fatty liver disease (NAFLD) is rising and has been reported as 20-30% in western populations [6]. It is also being increasingly recognised in the IBD population with a similar prevalence in this group [7,8]. The gold standard for diagnosis of NAFLD is liver biopsy [12]; we therefore accept that using ultrasound to look for evidence of fatty infiltration of the liver is a limitation of our study. However, ultrasound has been shown to be a useful tool for the detection of liver steatosis, allowing reliable and accurate results. The positive likelihood ratio and negative likelihood ratio of ultrasound for the detection of moderate-severe fatty liver, compared to histology, were 13.3 and 0.16 respectively [13,14].

We included ultrasound imaging within 5 years of commencing azathioprine to detect hepatic steatosis. It is therefore possible that azathioprine caused hepatic steatosis and an ALT rise in susceptible individuals. A prospective study would be required to assess this further.

With regard to our data, we found a prevalence of fatty liver of 32.2%, which is on the higher than expected range. However, this may be accounted for by our selection of patients who have required investigation of the liver with hepatic ultrasound. Three patients in the hepatic steatosis group had diabetes mellitus, compared to none in the group with a normal liver (P=.01). Mean BMI in the steatosis group was 31.9kg/m^2 compared with 23.6kg/m^2 in the no steatosis group (P<.001). The mean age was 47 years for those with steatosis compared to 31 years for those without (P<.001). Diabetes mellitus, obesity and advancing age are known risk factors for NAFLD consistent with those patients having pre-existing fatty liver disease [15].

Patients were excluded if there was evidence of any other liver pathology on USS or those with evidence of pre-existing hepatitis on blood tests. In our case no patients were excluded at this point. This was done with the intention of showing that hepatotoxicity was directly related to immunosuppression and not related to any other cause. It is not possible to say for certain that the hepatitis is the direct result of immunosuppression without a detailed history of alcohol and other substances causing hepatotoxicity, performing a full non-invasive liver screen to look for other

infective, inflammatory or autoimmune conditions and performing a liver biopsy. However, in the absence of any diagnosed, underlying liver pathology we suspect any identified hepatotoxicity is a direct result of azathioprine.

It is important to note that the incidence of hepatotoxicity in relation to IBD immunosuppressive therapies varies depending upon the definition of hepatotoxicity, but is reported as being between 0 and 17% [3,4,16]. In this study we looked specifically at ALT as a marker of possible hepatotoxicity and used any elevation in ALT to indicate hepatotoxicity. The average rise in ALT in patients with radiological hepatic steatosis was 8.19U/L. We accept that this does not necessarily represent a clinically significant rise. However, in patients with no ultrasound evidence of liver steatosis the average change in ALT was –1.20U/L and the P value for the difference between the two groups was <.001 which is therefore a statistically significant numeric difference between the groups.

The clinical relevance of elevations in liver enzymes in IBD is a matter of debate. However, as even mild elevations in ALT may be associated with liver injury and mortality from liver disease, close monitoring of liver biochemistry in patients being treated with azathioprine is recommended [17,18,19,20].

It has previously been reported that MMP is associated with hepatotoxicity at levels above 5700pmol /8x10^8 cells [4]. In our study there were a range of MMP levels across the 2 groups with 4 patients from each group having an MMP level of >5700 at 6 weeks. The median MMP levels recorded were 1859 pmol /8x10^8 cells in the liver steatosis group and 967 pmol /8x10^8 cells in the radiologically normal liver group (P=.04). This may suggest that patients with hepatic steatosis metabolise azathioprine differently, resulting in higher levels of MMP. The median TGN level for both groups was within the therapeutic range with a P value=0.40 suggesting no statistically significant difference between the two groups.

Average dosing of azathioprine across both groups was 1.88mg/kg which is slightly lower than the recommended therapeutic dose of 2.5mg/kg. The mean in patients with radiological steatosis was 1.73mg/kg compared to 1.95mg/kg in the group with a radiologically normal liver. This suggests that the higher metabolite levels were not due to higher dosing, but to differences in metabolism.

The linear regression analysis found strong evidence of an interaction between presence of hepatic steatosis, MMP levels and change in ALT (*P*<.001).

It may be that non-alcoholic fatty liver disease is a first hit for promoting liver injury in IBD patients, making them more susceptible to a high level of MMP (a second hit). Our data suggests that the hepatotoxic effects of high levels of MMP may only be relevant in those patients with hepatic steatosis. However, as a single centre study, only four patients in each group had MMP levels >5700pmol/8x10^8, a concordant study containing more patients with high MMP would be needed to confirm this finding.

Our study is consistent with previously published research suggesting that non-alcoholic fatty liver disease is a risk factor for the development of hepatotoxicity in IBD patients on thiopurines. We have gone further to implicate MMP in this process concluding that patients with hepatic steatosis may be more sensitive to MMP and may metabolise azathioprine differently. As the options for the

management of IBD remain finite and the prevalence of NAFLD continues to rise, improving our understanding in this area in order to inform treatment strategies is critically important.

Acknowledgments

All authors contributed substantially to data collection, interpretation of results and/or preparation of the manuscript. Dr Chris Penfold was the statistician who carried out the statistical analysis.

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